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Morphological studies of seeds of some subgenus (subgen. *Trifolium*, subgen.*Galearia*) of *Trifolium* L.

A.M. Asgarov*, K.A. Mammadyarova

*Genetic Resources Institute, Azerbaijan National Academy of Sciences, 155 Azadlig ave., 155, Baku AZ 1106, Azerbaijan; *For correspondence: askerov1@mail.ru*
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For the first time, micromorphological features of seeds of 6 species of clovers (*Trifolium* L.) of the Talysh flora, collected from the Lankaran-Lerik region of the Azerbaijan Republic, belonging to 2 subgroups and 4 sections were studied on an electron microscope (SEM). The shape of the seeds, their size, color, surface structure, the shape of hilum and their sizes are important taxonomic features. The structure of seeds of the studied species refers to 4 types: seeds with granular surfaces; seeds with tuberculate surfaces; seeds, which have a surface with crystal-like ledges. Features of the structure of the spores can be used, when specifying the status of subgenera; and sections, the remaining morphological features - when determining species of the genus of clover.

Keywords: *Talish, clover, seed, micromorphology, taxonomy*

INTRODUCTION

The genus *Trifolium* L. belongs to the legume family (*Fabaceae*) and includes 255 species (Zohary, 1984). Species of the clover are valuable forage, medicinal, green manure, and also honey plants.

In the Caucasus, 59 species of clover species are widespread (Grossheim, 1952), 43 species of them are found in Azerbaijan (Khalilov, 1952). According to the latest data, there are 50 species of genus *Trifolium* L. s.l. in Azerbaijan (Asgarov, 2016; Gurbanov, Mamedyarova, 2018). According to the same authors data, among the 5 large regions of Azerbaijan, the region with the largest number of clover species is Lankaran-Lerik region-37-39 species, or 76% of the clover species of the whole Azerbaijan. Species that are not found in Talysh mainly belong to the subgenus of *Lotoidea* Grantz. They are widespread in the Caucasus in the higher-altitude floristic complexes that are absent in Talysh.

Lankaran-Lerik region includes 4 botanical and geographical regions: Lankaran lowland, mountainous areas of Lankaran, Diabar (Zuvand) and Lankaran Mugan. In these areas, the spread of clover species is irregular. The most common types of clover spread in the mountainous areas of Lankaran (25 species).

In Flora of Azerbaijan (Khalilov, 1954) subgenera and sections were not indicated. Subgenera of *Trifolium* were adopted by E. Bobrov (1987), A. Grossheim (1954), some of which were later adopted as genus (Bobrov 1987; Roskov, 1990; Askerov, 2016).

Species of *Trifolium* L. of the Lankaran-Lerik region belongs to 4 subgenera and 10 sections. The largest number of species belong to the subgenusof *Trifolium* L. s.str.andcontains 27 species.

It should be mentioned that the statuses of a number of species of the Lankaran-Lerik region and Azerbaijan as a whole are contentious. In addition to general morphological research methods, they were specified by karyological, anatomical and other methods. This paper presents the results of a study of this issue by applying the micromorphological method when using the SEM microscope.

MATERIAL AND METHODS

Materials for our research are the seed samples collected by us during the expedition in the Lankaran-Lerik region in 2017-2018. We also used in our work samples of seeds conserved in the Genebank of the Genetic Resources Institute of the National Academy of Sciences of the Repub-

lic of Azerbaijan. Nomenclature changes were specified according to the monograph “The genus *Trifolium*” (Zohary et. al., 1984) and on the work of A.M.Asgarov (Flora of Azerbaijan, 2016), as well as The International Plant Names Index (IPNI).

For the analysis, samples of seeds on 6 species of clover collected from different populations were taken (*T. angustifolium*, *T. pratense*, *T. lappaceum*, *T. repens*, *T. resupinatum*, *T. tumens*). The collected material was placed in special sterile paper bags and, under laboratory conditions; the moisture was removed by applying silica gel.

Under the scanning electron microscope, (2-3) samples of mature seeds from each species were taken; the seeds were placed on previously prepared tables covered with adhesive carbon tape. Seed samples are sprayed with metal for 1-2x minutes on a JEOL JFC1600 ion sprayer unit. Seed samples were studied from the side surface. The surface of the seeds is photographed with a JEOL JSM6610 lv electron microscope at 4000 times magnification. Under the electron microscope, various structures of the seed surface, the length, and width of the hilum (ribbed) were studied.

RESULTS AND DISCUSSION

Species of *Trifolium* L.in the Lankaran-Lerik region belongs to 4 subgenera and 10 sections. The largest number of species belonging to the subgenus *Trifolium* L. s.str. and contains 27 species.

The statuses of a number of clover species of the Lankaran-Lerik region and Azerbaijan as a whole are contentious. In addition to general morphological research methods, they were specified by karyological, anatomical and other methods. This paper presents the results of a study of this issue by applying the micromorphological method when using the SEM microscope.

Taking into account the morphological features of the seeds and their structural data, a detailed description of 6 species of clovers of the Lankaran-Lerik region is given below. During describing the section, were used the works of M.Zohary et D.Heller and E.G. Bobrova (1987).

Sect. *Stenostoma* Gibelli et Belli

The calyx is closed as a slit with callous thickening in the throat. Corolla deciduous, usually whitish-yellow, rarely pink.

From this section, morphologies of spores of a single species, collected during an expedition of 2018, were studied.

***T. angustifolium* L.**

An annual plant, appressed-hairy, the stem is stiffly erect, and sometimes procumbent, branched, 10-30 cm high. Seeds are ellipsoidal, 0.9-2.2 mm, the surface is smooth, brown. The surface structure is tightly granular. The hilum is bean-shaped, long 0.12 mm. 2n = 14, 16 (Table 2, Figure 1).

They spread in Europe, the Mediterranean, in the countries of South-West Asia. In Azerbaijan, it grows mainly in all regions, and in the Lankaran-Lerik region - in the Lankaran lowland and the Lankaran mountain region (Table 1).

Sect.*Trifolium*

One species was studied. Calyxin the throat without a callous thickening, but with a ring of thick hairs, and sometimes with an annular leathery fold. The corolla is deciduous, with different red or pink, rarely whitish shades.

***T. pretense* L.**

A perennial plant, usually hairy, branched stem, plant height is 15-40 cm. The seeds are ellipsoid or ovate, asymmetrical cordiform or reniform, brown, almost smooth surface, 1.7-2.3 x 1.2-1.5 mm, light or dark, yellowish green, have gray-brown and purple. The surface structure is large-granular. The hilum is oblong-spherical, length - 0.08 mm. 2n = 14 (Table 2, Figure 1).

The plants spread in Europe, the Mediterranean and in Asian countries. They are found in all regions of Azerbaijan, including Lankaran-Lerik region.

It is a valuable forage and medicinal plant (Table 1).

***T. lappaceum* L.**

Annual plant. Stems are often prostrate, hairy, 10-40 cm height. Seeds are oval, hard, slightly shiny, dark brown, 1.2-1.4 x 1-1.2 mm. The structure of the surface with tightly semicircular ledges (forms a network). Hilums are oblong-oval, length 0.14 mm. 2n = 16 (Table 2, Figure 2).

They spread in South-Eastern Europe, the Mediterranean, South-Western and Central Asia. It is found in all regions of Azerbaijan, including Talysh (Table 1).

Table 1. Research species, their location and coordinates

Subgenus	Sections	Species	Collection place and coordinates	Date of collection
I. *Trifolium* L. s. str.	1.*Stenostoma*	*T. angustifolium*	Djalilabad region, Zahmedabad village N39^0 14.954' E048^0 27.420' A 508 m	16.05.2017
	2. *Trifolium*	*T. pratense*	Guba region, Geshresh village N41^0 11.333' E48^0 28.754' A 647 m	13.07.2016
		T. lappaceum	Djalilabad region,, Soltankend village N39^0 41.6' E48^0 16.21' A 536 m	15.06.2017
	3.*Lotoidea*	*T. repens*	Lerik region, Divagach village N38^0 40.014' E048^0 21.663' A 1470 m	03.07.2018
II. *Galearia* (C. Presl) Hossain.	4.*Galearia*	*T. resupinatum*	Lerik region, Lyulakaran village N38^0 74.681' E48^0 39.598' A1300 m	19.05.2017
		T. tumens	Djalilabad region, Zahmedabad village N39^0 14.954' E048^0 27.420' A 508 m	16.05.2017

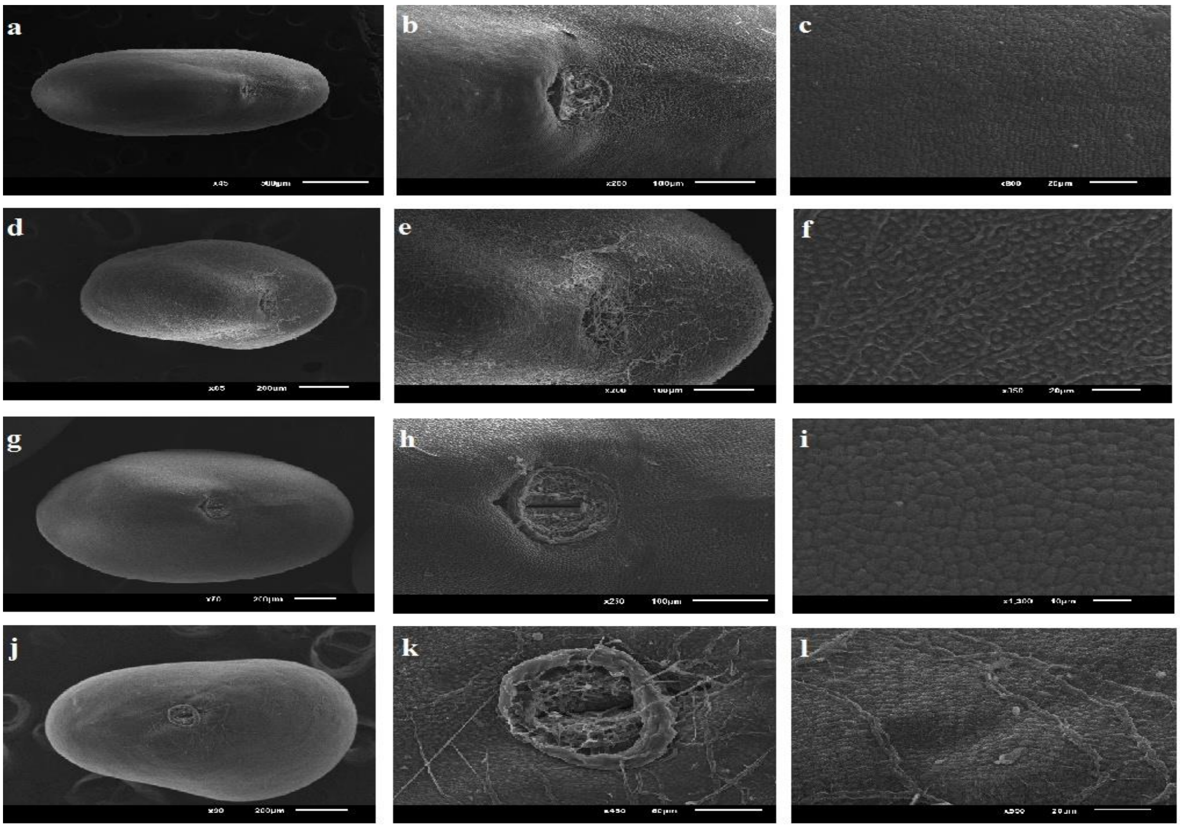


Fig. 1. Shape and structure of seeds under the scan electron microscope (SEM): a – c *T. angustifolium*; d – f *T. repens*; g – I *T. pratense*; j – l *T. tumens.*

Table 2. Seed morphological features

Species	Size(mm) (min-max)	Shape	Color	Surface shape	Surface structure	Hilum forms	Hilum length (mm)
T. angustifolium	0.9-2.2 x 0.7-2.0	ellipsoidal	brown	smooth	tightly granular	bean shaped	0.12
T. repens	1.1-1.5 x 0.9-1.2	cordi or reniform	yellow, yellowish-pink	smooth andshiny	rarely granular	oblong-bean shaped	0.08
T. pratense	1.7-2.3 x 1.2-1.5	ellipsoid or ovate	brown	smooth	large-granular	oblong-spherical	0.08
T. tumens	0.7-1.1 x 0.5-0.9	egg-shaped	white-yellowish	smooth	Densely tuberculate	spherical, indented laterally	0.12
T. resupinatum	1.3-1.8 x 0.9-1.3	ellipsoid or oval	light or dark	smooth	Smooth, semi-annular ledges, forms a network	oval	0.12
T. lappaceum	1.2-1.4 x 1-1.2	oval	dark brown	smooth	tightly semicircular ledges, forms a network	oblong-oval	0.14

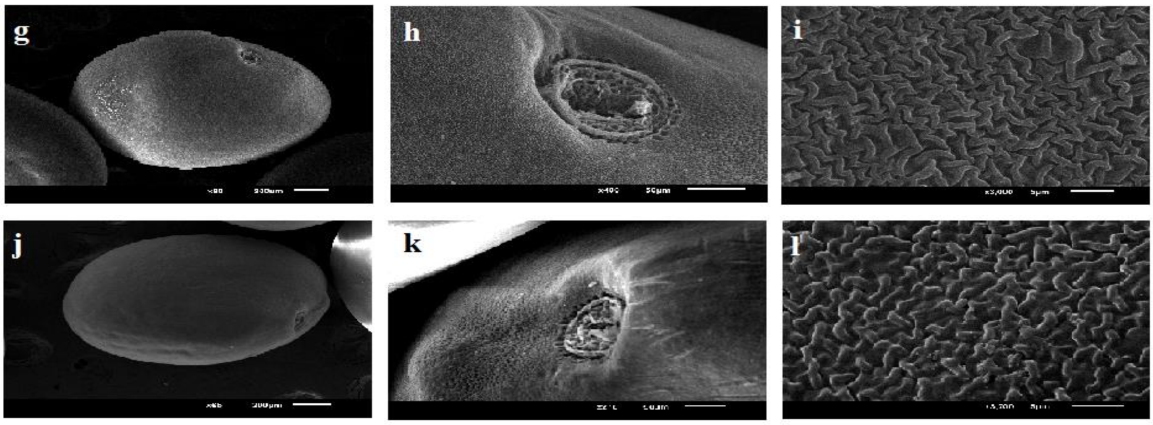


Fig. 2. Shape and structure of seeds under the scan electron microscope (SEM): g – I *T. resupinatum*; j – l *T. lappaceum*.

From this section, the morphology of spores of one species was studied.

Sect. ***Lotoidea*** **Grantz.**

All flowers on peduncle 0.5-3mm long, with well-developed bracts. Calyx with 5 lanceolate or narrow-lanceolate teeth, 2 upper teeth are often longer, not inflated after flowering. The corolla is white,rarely pink or reddish, non-deciduous after flowering.

T. repens **L.**

A perennial plant, creeping stem, plant height is 10-20 cm. The seeds are cords or reniform, 1.1-1.5 x0.9-1.2 mm, the surface is shiny, smooth, immature seeds are yellowish-pink, mature seeds are of light brown. The surface structure is rarely granular. The form of hilum seeds are oblong-bean-shaped, length 0.08 mm. 2n=32 (Table 2, Figure 1).

They spread in Europe, the Mediterranean and in Asian countries. They are found in all regions of Azerbaijan, including Lankaran-Lerik region. It is a valuable pasture and forage plant (Table 1).

Sect. ***Galearia*** **(C. Presl) Godr.**

All blossoms on peduncles up to 1mm long., and located in the axils of the bracts. The calyx is two-lipped; the upper lip is systiform-swelling, membranous during bearing. The corolla is pink, pink-white or reddish, usually deciduous after flowering. From this section, the morphology of two types of spores was studied.

T. resupinatum **L.** - An annual or perennial plant, the stem is branched, bare, plant height is 10 - 40 cm. Seeds ellipsoid or oval, asymmetrical cordiform 1.3-1.8 x 0.9-1.3mm., surface smooth, light or dark, dark yellow or brownish - yellowish, in mature, the seeds are pinkish or blackish.

The structure of the surface with rarely semi-annular ledges (forms a network). The hilum is oval, the length is 0.12 mm. 2n = 16 (Table 2, Figure 2).

Distributed in Asian and Mediterranean countries. It is found in all regions of Azerbaijan, including Talysh. It is a valuable forage plant (Table 1).

T. tumens **Steven ex M. Bieb.**- A perennial plant, the stem is bare, erect or creeping, height is 10 - 40 cm. Seeds are egg-shaped, 0.7 - 1.1 mm.

The surface is smooth, white-yellowish. The surface structure is densely tuberculate. Hilum is spherical, indented laterally, 0.12 mm long. 2n = 16, 32 (Table 2, Figure 1).

This species spread in South-West Asia (Azerbaijan, Georgia, Armenia, Turkey, and Iran). It grows in all regions of Azerbaijan and Talysh (Table 1).

Thus, researches were conducted on 6 species of clover belonging to 4 sections and 2 subgenus, collected from different populations of the Lankaran-Lerik region.

As a result of the analysis, obtained by us on the study of micromorphological features of seeds, their 3 morphological types were found. The particular focus is paid to the surface features of the seeds and their structural features.

1. Seeds with smooth, covered with dense granules surface. This includes *Trifolium angustifolium*, from section *Stenostoma*; *T. repens*, from section *Lotoidea* and *T. pretense* from section *Trifolium*

2. Seeds whose surface is smooth, densely “tuberculate”. This includes *T. tumens* from the *Galearia* section.

3. Seeds with smooth, annular ledges surface. These ledges are tightly annular (forms a network).

This type of seed is characterized by peculiar characteristics and include two types:

T. resupinatum from section *Galearia* and T. *lappaceum* from section *Trifolium.*

According to the micromorphological descriptions of seeds of certain types of clovers, their shapes and sizes (oval, heart-shaped, bud-shaped, etc.), color (brown, yellow, black, etc.), forms and sizes of hilum (bean-shaped, oval, spherical, e) are highly variable and characteristic for the description of species.

And such features of seeds as the shape of their surface, as well as their structural features are valuable in the description of the section and subgenera.

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Trifolium L. cinsinin bəzi yarımcinslərinə (subgen. *Trifolium,* subgen. *Galearia*) aid növlərin toxumlarının morfoloji öyrənilməsi

A.M. Əsgərov, K.A. Məmmədyarova

AMEA Genetik Ehtiyatlar İnstitutu

Üçyarpaq yonca (*Trifolium* L.) cinsinin 2 yarımcinsinə (subgen. *Trifolium,* subgen. *Galearia*) aid növlərinin toxumlarının mikromorfoloji xüsusiyyətləri skan elektron mikroskopunda tədqiq edilmişdir. Öyrənilən növlərin 3 toxum tipinə aid olması müəyyən edilmişdir. Aşkar edilən konstant əlamətlərdən yarımcins və növlərin təyinində və onların statuslarının dəqiqləşdirilməsində istifadə oluna bilər.

Açar sözlər: *Talış, üçyarpaq yonca, toxum, morfologiya, taksonomiya*

Морфологическое изучение семян некоторых видов из подродов (subgen. *Trifolium,* subgen. *Galearia*) рода *Trifolium* L.

А.М. Аскеров, К.А.Мамедярова

Институт генетических ресурсов НАН Азербайджана

На сканирующем электронном микроскопе исследованы микроморфологические особенности шести видов клевера (*Trifolium* L.), относящихся к двум подродам (subgen. *Trifolium,* subgen. *Galearia*). Установлено, что признаки изученных видов клевера относятся к трем морфологическим типам семян. Обнаруженные константные морфологические признаки семян клевера (*Trifolium* L.) могут быть использованы при определении подродов и видов, а также в уточнении их статусов.

Ключевые слова: *Талыш, клевер, семена, морфология, таксономия*